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14. ABSTRACT
In this proposal, we aim to test the hypothesis that complete AR pathway inhibition selects for subpopulations of tumor cells that are completely independent of AR signaling and further, that these resistant cells will have activated---and be dependent upon---a limited set of specific survival and growth regulatory pathways (stemming from genomic alterations in specific oncogene networks) that can be identified and targeted. The proposal comprises three Specific Aims: Aim 1 will define the genomic alterations and transcript variants that comprise 'states' of ARIPC. Aim 2 will determine if targeting/inhibiting the survival pathway(s) that emerge following AR pathway ablation will restrain tumor growth. Aim 3 will determine if simultaneously co-targeting the AR pathway and ARIPC survival pathway(s) in AR-sensitive prostate cancers will augment tumor responses and delay/prevent recurrences. During this funding period we have: (i) completed laser capture microdissection of CRPC prostate cancers to acquire RNA and DNA; (ii) completed transcript profiling for 196 CRPC metastasis; (iii) completed genomic analyses for 196 prostate cancer metastasis; (iv) identified a program of AR-repressed genes that may influence the development of CRPC; (v) completed preclinical studies of two putative AR-bypass pathways. Initiated integrated molecular analyses and high-throughput gene knockdown screens of advanced metastatic CPRC to identify additional molecular pathways that operate to promote prostate cancer growth in the absence of AR signaling.

15. SUBJECT TERMS
prostate cancer, androgens, androgen receptor, molecular profiles

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Introduction

This project aims to exploit data generated in the Nelson and Febbo groups demonstrating that although most CRPC tumors exhibit androgen receptor (AR) activity, there is significant heterogeneity in the level of AR activity and some cancers appear to have trivial or no AR activity ("AR-null"). Importantly, even in tumors that demonstrate global continued AR activity, there are infrequent tumor deposits with no AR expression. Thus, with the application of increasingly potent blockade of the androgen axis, resistant clones/tumors exhibiting an "AR-null" signature have emerged and a greater percentage of patients have AR-null CRPC. However, at this time, it is unclear what phenotypes, genotypes, and attendant dominant growth and survival pathways will be operative to "drive" an AR-null prostate malignancy.

The objective of this proposal is to test the hypothesis that complete AR pathway inhibition selects for subpopulations of tumor cells that are completely independent of AR signaling and further, that these resistant cells will have activated---and be dependent upon---a limited set of specific survival and growth regulatory pathways (stemming from genomic alterations in specific oncogene networks) that can be identified and targeted. The research plan comprises 3 Specific Aims to test these hypotheses. Aim 1 will define the genomic alterations and transcript variants that comprise 'states' of ARIPC. Aim 2 will determine if targeting/inhibiting the survival pathway(s) that emerge following AR pathway ablation will restrain tumor growth. Aim 3 will determine if simultaneously co-targeting the AR pathway and ARIPC survival pathway(s) in *AR-sensitive prostate cancers* will augment tumor responses and delay/prevent recurrences.

Body

This proposal was designed as a "synergy" project between the laboratories of Dr. Phil Febbo at the University of California, San Francisco (UCSF) and Dr. Peter Nelson in the Division of Human Biology at the Fred Hutchinson Cancer Research Center (FHCRC). Because these are separate awards to the two investigators, this progress report is specific to tasks from the statement of work (SOW) assigned to the Nelson Lab only (or to progress within the Nelson Lab for joint tasks). As per the instructions, progress is reported in association with each of the relevant tasks listed in the SOW. To complete the Project Aims, we have divided the proposed studies into discrete Tasks. We have numbered these Tasks (e.g. Task 1, Task 2, etc) and have designed them as **N** for Nelson lab, **F** for Febbo lab, and **NF** for Nelson and Febbo joint task. The time frame for the Task is noted as Year (Y1-3) and Quarter (Q1-4). Progress:

Task 1. Animal protocol administration

*1a: Submit established animal protocol to DOD for review (Y1Q1)-**N***

Task complete.

*1b Respond to comments from DOD Review (Y1Q1)—**N***

Task complete and protocol approved.

*1c Submit revised final animal protocol to Institutional IACUC approval (Y1Q1)—**N***

Task complete and protocol submitted.

1d Respond to comments from IACUC (Months 7) (Y1Q2)—N

Task complete and protocol amended.

Milestone #1: IACUC Approval of amended animal protocol (Y1Q3)
Milestone complete and protocol approved. FHCRC IACUC #1775

Milestone #2: Activate Amended Animal Protocol (Y1Q3)
Milestone complete and protocol activated.

1f. Amend/renew animal protocol to include testing of novel pathways (Y2Q2)—N
Milestone complete and protocols activated.

1g. Annual renewal of animal protocol (Y3Q3)—N
Milestone complete and protocols activated.

Task 2: Analysis of scientific aim 1: Integration of genome-scale bioinformatics-based approaches with quantitative assessments of gene expression measurements to define the pathways associated with ARIPC

2a. Laser Capture Microdissection of 150 CRPC specimens (Y1Q3)—NF
We have focused this task on 150 CRPC metastatic prostate cancer samples. These were acquired primarily through the rapid tissue acquisition necropsy program and represent single or multiple metastasis from the same patient. *We have now expanded this aim to include 196 prostate cancer metastasis and primary tumors.* Laser Capture Microdissections were performed on each of these 196 tissues to enrich for tumors and appropriate matched cell counterparts and exclude benign components (see details in previous progress report). High quality RNA and DNA was obtained from 196 samples and used for molecular profiling (see tasks below).

2b. (Revised) Splice variant analysis of 150 CRPC specimens (Y1Q4)—F
Task to Dr. Febbo.

2c. (Revised) SNP/Genomic Copy Number analysis of 150 CRPC specimens (Y1Q4)—N
We have completed array CGH analyses of 196 tumors and matched benign (constitutional) DNA to determine copy number variation across the prostate cancer genome and followed this up with targeted sequencing to determine single nucleotide polymorphism (SNP) and mutation status. Figure 1 shows the results of the copy number variation analysis (loss/gain) across the 196 prostate samples. Recurrent high frequency alterations in several loci are shown including amplification of the androgen receptor (AR) and alterations in several genes that may contribute to AR Pathway Independent Prostate Cancer (ARIPC) (**Figure 1**).

Milestone #3: Complete processing of 150 CRPC specimens (Y1Q4)
Task completed. See above.

2d. (Revised) Classify each CRPC with respect to AR splice variation (Y1Q3)F
Task to Dr. Febbo.

2e. (Revised) Classify each CRPC with respect to AR amplification (Y1Q3)F
Task to Dr. Febbo.

2f. (Revised) Classify each CRPC with respect to AR activity, androgen levels, AR splice variation, and AR copy number (Y1Q4)F
Task to Dr. Febbo.

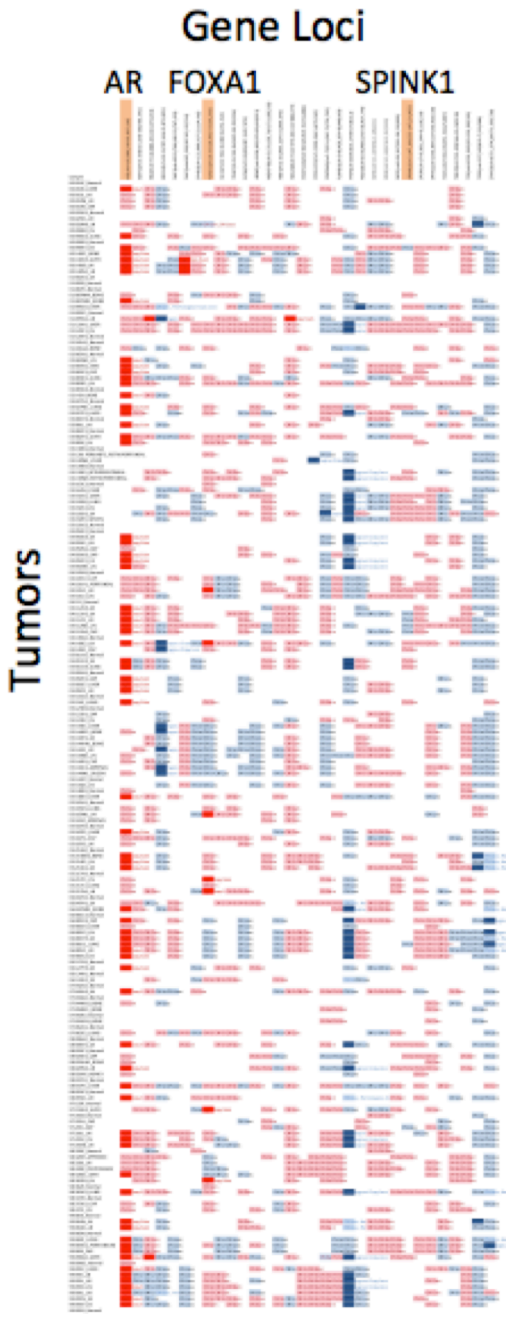

Figure 1. Copy number analyses of prostate cancer metastasis. Red depicts chromosomal regions with copy number gain and Blue depicts regions with copy number loss. X-axis (columns) of figure are selected gene loci with recurrent alterations across the prostate cancer metastasis. The Y-Axis (Rows) are individual tumors or matched benign tissue (constitutional DNA). AR and FOXA1 are commonly altered in AR-driven CRPC. SPINK1 amplification represents a potential pathway promoting ARIPC.

Milestone #4:(Revised) Impact of AR splice variation and AR genetic amplification on classification of CRPC specimens into AR-specific states (Y1Q4)

This aim/milestone is continuing but is not yet complete. As described in the Y1 progress report, we continue to classify the prostate cancer metastasis based on gene expression profiles into AR activity categories and are now subdividing the AR activity 'high' category, representing the >90% of the tumors, into subcategories based in AR splice variant status and AR amplification status. The preliminary results indicate that the major if AR activity 'high' category also has AR amplification, ~60%. Completion of AR splice variant analysis is pending. A focal point of the ongoing studies is the identification of AR-null tumors that do not exhibit a neuroendocrine/small cell phenotype, as this is a known well-described variant of ARIPC and the objective of the present study is to identify alternative 'bypass pathways' that do not follow this established phenotype. In addition to the microarray classifications, we are including immunohistochemical studies to rule-out neuroendocrine differentiation. **Figure 2** demonstrates several ARIPC tumors that are AR-null and that do not exhibit NE differentiation as determined by lack of chromogranin and synaptophysin expression.

2g. Associate additional pathways with ARIPC. (Y1Q4)—Task to Dr. Febbo

In addition to studies conducted by Dr. Febbo in defining additional pathways associated with ARIPC, we reported in the Y1 Progress Report the identification of a pathway involving LSD1 and H3K4me1,2 demethylation in modulating AR activity. This pathway may represent a ligand-independent subtype of CRPC, still dependent on AR signaling, but does not appear to promote or represent a driver of ARIPC (see (Cai, He et al. 2011)).

	IHC Results			
Tumor ID	AR	PSA	ChA	Syn
01-046 A2	0	0	0	0
01-046 F2	0	0	+	+
01-046 G2	0	0	+	+
01-095 I2	0	0	0	0
03-026 GG1	0	+	N/A	N/A
03-026 R2	0	+	N/A	N/A
03-026 S3	0	+	N/A	N/A
03-026 T2	0	+	N/A	N/A
03-026 W1	0	0	+	+
03-163 N1	0	0	0	0
04-112 M2	0	0	0	0
05-116 H1	0	+	N/A	N/A
98-362 BC	0	+	N/A	N/A
98-380 HC	0	+	N/A	N/A
98-380 VA	0	0	+	+
98-380 VE	0	0	+	+
98-388 A	0	+	N/A	N/A
98-388 ID	0	+	N/A	N/A
98-388 QA	0	+	N/A	N/A
98-390 GB	0	+	N/A	N/A

Figure 2. IHC of metastatic prostate cancers showing AR and PSA expression (AR activity) and NE-cell differentiation. ChA is chromogranin A; Syn is synaptophysin.

To identify genes capable of promoting the growth of prostate cancer cells in the absence of exogenous AR ligands, we performed a high-throughput RNAi screen (HTRS) using two androgen-sensitive prostate cancer cell lines; LNCaP and VCaP. We hypothesized that a subset of genes and gene networks could confer a castration-resistant phenotype in prostate cancer cells previously dependent upon androgen-mediated signaling for growth and survival.

Briefly, cells were cultured in charcoal-dextran stripped fetal bovine serum (CSS) in order to simulate androgen deprivation therapy. A library of siRNAs designed to inhibit the translation of 6650 individual genes (designated the 'druggable genome') was used in an arrayed screening strategy whereby a pool of three siRNAs targeting each specific gene was separately introduced into replicate cell cultures (one gene per well) in 384-well culture plates. After 96 hours of growth, cell numbers were estimated using the Cell Titer-Glo luminescence reagent. Raw luminescence signal intensity from the HTRS screen was transformed into Z-scores within each plate, and median standardized Z-scores for each gene were used in downstream analysis. To prioritize genes for further study, we arbitrarily set a significance threshold of Z ≥1.96. At this cut-point, suppression of 380 unique genes in LNCaP and 240 unique genes in VCaP induced cell growth in the absence of exogenous androgens (**Figure 3**), of which 40 induced growth in both cell lines. Of the 40 genes whose suppression induced growth in both LNCaP and VCaP cells, 2 encoded components of the PP2A serine and threonine phosphatase complex: PPP2R2C and PPP2R1A.

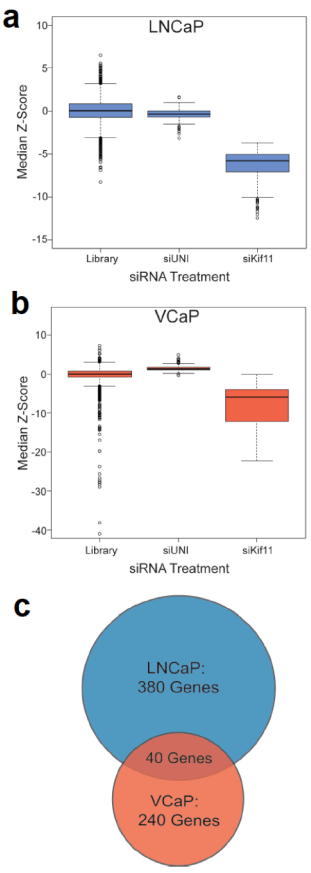

Figure 3. High throughput RNAi screening identifies suppressors of CRPC growth. Pools of siRNAs targeting 6650 genes (Library) were used to knockdown individual gene targets in LNCaP and VCaP cells grown in androgen-depleted medium. HTRS Z-score results are plotted for the LNCaP (a) and VCaP (b) cell lines. siUNI is a non-targeting scrambled control siRNA. siKif11 is a positive control cell death-inducing siRNA targeting Kif11. (c) In the library screen, 380 siRNAs and 240 siRNAs induced castration-resistant growth in LNCaP or VCaP, respectively. Of these, 40 genes induced growth in androgen depleted conditions in both cell lines (Significance threshold: Z≥1.96).

2h. Validate additional pathways with ARIPC (Y2Q4)—NF

To prioritize HTRS hits exhibiting potential tumor-suppressor expression patterns, we cross-referenced the screen results with transcript abundance levels determined by microarray measurements of laser-capture microdissected benign prostate epithelia (n=15), ADT-

resistant primary prostate tumors (n=14), and CRPC metastases (n=54). Out of the 40 proliferation-suppressor genes identified in the *in vitro* HTRS, 14 genes were significantly downregulated in primary and metastatic CRPC samples compared to benign prostate epithelia, including PPP2R2C and PPP2RA1 (**Figure 4**). Specifically, the mean expression of PPP2R2C in these primary and metastatic cancers was 3.85-fold lower (P=0.034) and 5.69-fold lower (P<0.001) than benign prostate epithelia, respectively. PPP2R1A expression was also decreased in metastatic prostate tumors (1.55-fold, P<0.001).

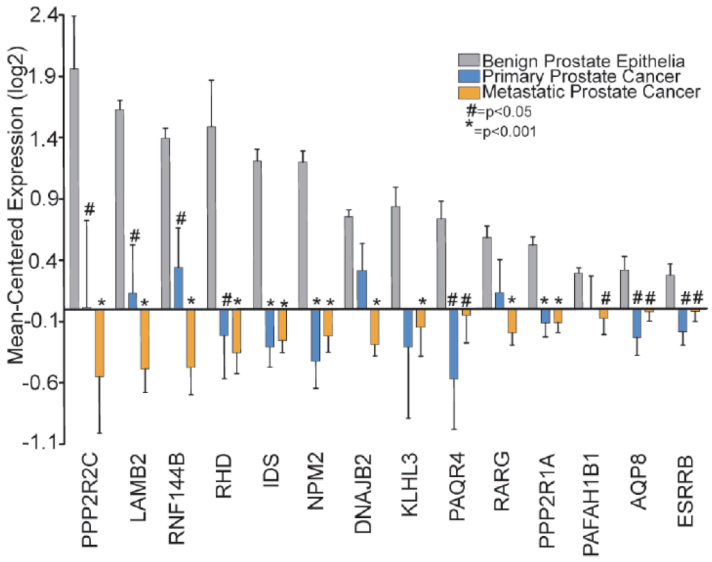

***Figure 4. PPP2R2C is downregulated in primary and metastatic prostate cancer*. Gene expression microarray data from 16 benign prostate samples, 15 ADT-resistant primary tumors, and 55 CRPC metastases was cross-referenced with results from the HTRS experiments. Of the 40 overlapping hits identified in the VCaP and LNCaP HTRS experiments, 14 were significantly downregulated in primary tumors and CRPC metastases compared to benign prostate epithelia. Statistical significance was determined by the Student's T-test.**

We next evaluated HTRS experimental results and human prostate gene expression data for evidence of other PP2A components behaving as tumor suppressors. Previous studies have reported that suppression of the PP2A constituents PPP2CA and PPP2R2A can promote castration-resistant prostate cancer cell growth (Singh, Bafna et al. 2008, Mao, Boyd et al. 2011). However, transcripts encoding these PP2A subunits were not down-regulated in the primary prostate cancers we evaluated. Further, transcripts encoding PPP2CA were significantly higher in metastatic CRPC (1.9-fold, p<0.001. In the HTRS experiments, siRNAs targeting PPP2CA or PPP2R2A did not induce significant castration-resistant growth in either LuCAP or VCaP cells.

Milestone #5: Identifications of additional pathways associated with ARIPC (Y2Q2)

Through the gene expression profiling studies and the RNAi screening studies we have identified several genes and pathways with the potential to modulate ARIPC growth. These include MYC, HDACs, LSD1, PPP2R2C, Fibroblast growth factors and others listed in **Figure 5**. Further confirmation of the effects of modulating components of these pathways is underway under Task 3.

Figure 5 (Below). Genes with significant growth suppressing effects in the context of CRPC. Suppression of these genes with siRNA promoted the growth of prostate cancer cells in the absence of AR ligands.

Genes Promoting ARIPC Growth.

Official Gene Symbol	Gene ID	Gene Name	Median Z-Score	
			LNCaP	VCaP
APOB	338	Apolipoprotein B (including Ag(x) antigen)	2.43	1.97
AQP8	343	Aquaporin 8	2.40	2.26
ART4	420	ADP-ribosyltransferase 4	2.03	2.72
CAPN13	92291	Calpain 13	2.11	2.29
CYP2A6	1548	Cytochrome P450, family 2, subfamily A, polypeptide 6	2.19	2.15
DNAJB2	3300	DnaJ (Hsp40) homolog, subfamily B, member 2	3.79	2.09
DPEP1	1800	Dipeptidase 1	3.22	2.14
ESRRB	2103	Estrogen-related receptor beta	2.51	2.81
GABRA2	2555	Gamma-aminobutyric acid A receptor, alpha 2	2.44	2.53
GBP7	388646	Guanylate binding protein 7	3.07	2.86
IDS	3423	Iduronate 2-sulfatase	2.00	2.25
KCND2	3751	Potassium voltage-gated channel, Shal-related subfamily, 2	2.20	3.07
KCTD5	54442	Potassium channel tetramerisation domain containing 5	3.13	2.47
KCTD12	115207	potassium channel tetramerisation domain containing 12	3.54	3.81
KIF2C	11004	Kinesin family member 2C	2.01	2.01
KLHL3	26249	Kelch-like 3	2.39	2.54
KLHL13	90293	Kelch-like 13	3.16	2.42
LAMB2	3913	Laminin, beta 2	3.48	3.46
MGC26694	284439	Hypothetical protein MGC26694	3.49	7.26
MIPEP	4285	Mitochondrial intermediate peptidase	2.27	2.67
NPM2	10361	Nucleophosmin/nucleoplasmin, 2	2.26	2.43
NR2F1	7025	Nuclear receptor subfamily 2, group F, member 1	2.34	3.45
PAFAH1B1	5048	Platelet-activating factor acetylhydrolase, isoform Ib, alpha	2.70	2.20
PAQR4	124222	Progestin and adipoQ receptor family member IV	2.38	2.25
PLOD2	5352	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	2.32	3.09
PPP2R1A	5518	Protein phosphatase 2 regulatory subunit A (PR65 alpha)	2.20	3.29
PPP2R2C	5522	Protein phosphatase 2 regulatory subunit B (PR55 gamma)	2.81	2.10
PYGM	5837	Phosphorylase, glycogen; muscle	3.29	2.25
RAD23A	5886	RAD23 homolog A	3.21	2.04
RARG	5916	Retinoic acid receptor, gamma	2.04	2.05
RHD	6007	Rhesus blood group, D antigen	2.41	2.03
RNF20	56254	Ring finger protein 20	3.01	2.03
RNF144	9781	Ring finger protein 144	2.39	2.77
SRM	6723	Spermidine synthase	2.34	2.07
TNXB	7148	Tenascin XB	2.32	2.57
TOPBP1	11073	Topoisomerase (DNA) II binding protein	2.48	2.22
UBA6	55236	Ubiquitin-like modifier activating enzyme 6	2.01	2.09
UNC5C	8633	Unc-5 homolog C	3.68	3.18
USP21	27005	Ubiquitin specific protease 21	2.13	2.17
ZFAND3	60685	Zinc finger, AN1-type domain 3	3.83	3.65

Task 3: Analysis of scientific aim 2: To determine if targeting/inhibiting the pathway(s) associated with ARIPC will restrain prostate tumor growth.

3a.(Revised) Determine the impact of inhibiting MYC in ARIPC (Y2Q4)-N

The experiments involved in this task are continuing. Our preliminary studies indicate that Myc expression can promote the growth of prostate cancer cells in the absence of AR ligands (**Figure 6**). Ongoing experiments are evaluating whether Myc can completely bypass the requirement of AR and whether Myc suppression can suppress the growth of ARIPC.

3b. (Revised) Determine the impact of targeting HDAC family members in ARIPC (Y2Q4)—F

Task to Dr. Febbo.

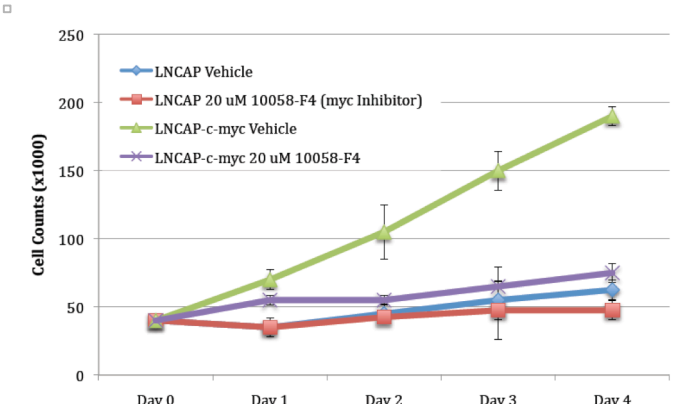

Figure 6. Myc expression promotes the growth of AR ligand dependent prostate cancer cells in the absence of AR ligands. The effect is blocked by the addition of the Myc inhibitor, 10058-F4.

Milestone #6: (Revised) Results demonstrating the impact of MYC and HDAC inhibition on ARIPC xenografts (Y2Q4)

Task to Dr. Febbo.

3c. Determine the impact on ARIPC growth of inhibition of candidate pathway #1 identified during Tasks 2g and 2h-(Y3Q3)NF

As four of the 16 PP2A subunits tested in the HTRS experiments induced AR ligand-independent proliferation in at least one cell line, we sought to further assess the mechanism(s) by which PP2A activity contributes to this phenotype. We selected PPP2R2C for further investigation based on the findings that PPP2R2C induced significant castration-resistant proliferation in both cell lines evaluated, and PPP2R2C was the HTRS hit found to be most down-regulated in castration-resistant metastatic prostate cancer compared to benign epithelium (**Figure 4**).

Because the HTRS experiments were performed using pools of three siRNAs targeting each gene, we evaluated individual siRNA efficacy by transfecting LNCaP and VCaP with the deconvoluted pool of PPP2R2C siRNAs, designated siRNA #1-3. Suppression of PPP2R2C by gene-specific siRNA was confirmed by qRT-PCR and correlated with assays of cell proliferation. PPP2R2C knockdown by si#2 and si#3 were confirmed at the protein level in both cell lines (**Figure 7a**). Scrambled control siRNAs (siUNI) did not alter PPP2R2C expression or influence cell proliferation (**Figure 7b**). The positive control for cell death, siKiff11, substantially reduced the number of tumor cells in both LNCaP and VCaP experiments (**Figure 7b**). As expected, exposing LNCaP or VCaP cells to androgen (R1881) stimulated proliferation and induced the AR target genes PSA, FKBP5, TMRPSS2, and ERG (in VCaP; **Figure 7b-d**). siRNA#1 suppressed PPP2R2C transcripts by 10-fold in both cell lines, but did not influence cell proliferation in either line. However, both siRNA #2 and siRNA #3 reduced PPP2R2C mRNAs in LNCaP by 11.1-fold and 4.2-fold (P<0.001), respectively (**Figure 7b-c**), and induced cell proliferation in androgen-depleted medium by 42% (siRNA #2, P<0.001) and 72% (siRNA #3, P<0.001). This increase in cell number approximated the influence of adding the androgen R1881 (**Figure 7b**). In VCaP cells PPP2R2C transcripts were reduced 6.7-fold by siRNA #2 and 4.2-fold by siRNA #3 (P<0.001; **Figure 7d**), and these siRNAs increased cell proliferation by approximately 33% in androgen depleted growth conditions (P<0.001; **Figure 7b**). PPP2R2C suppression did not alter AR or PSA expression in either cell line (**Figure 7c-d)**. Suppression of PPP2R2C with siRNA #2 in LNCaP cells did slightly decrease the expression of FKBP5 (1.4-fold, P=0.02) and TMPRSS2 (1.3-fold, P=0.02) and resulted in a 1.6-fold increase in ERG expression (P=0.01; **Figure 7c**). Suppression of PPP2R2C with siRNA #3 in VCaP resulted in decreased TMPRSS2 expression (1.2-fold, P=0.01; **Figure 7d**). PPP2R2C downregulated with si#3 in LNCaP and

si#2 in VCaP did not affect androgen-regulated gene expression. These data indicate that the growth advantages attained from PPP2R2C knockdown were not mediated by an increase in canonical AR transcriptional activity.

To further assess the potential role of the AR in PPP2R2C mediated prostate cancer cell growth in the context of androgen depletion, LNCaP and VCaP cells were treated with the AR antagonist MDV3100. In the absence of exogenous androgens, MDV3100 had a slight effect in reducing VCaP cell numbers after 96 hours (12%, p=0.05) and no discernable effect on LNCaP growth (**Figure 7e-f**). As expected, MDV3100 effectively suppressed the proliferative effects induced by the synthetic androgen R1881 in both lines (**Figure 7e-f**). Suppression of PPP2R2C induced cell proliferation in VCaP cells (si#2: 24%, p<0.001; si#3: 18%, p=0.002) and LNCaP cells (si#2: 32%, p<0.001; si#3: 40%, p<0.001) in androgen-depleted medium despite treatment with MDV3100 (**Figure 7e-f**). Collectively, these experiments confirm that PPP2R2C loss is sufficient to induce AR pathway-independent proliferation in prostate cancer cells.

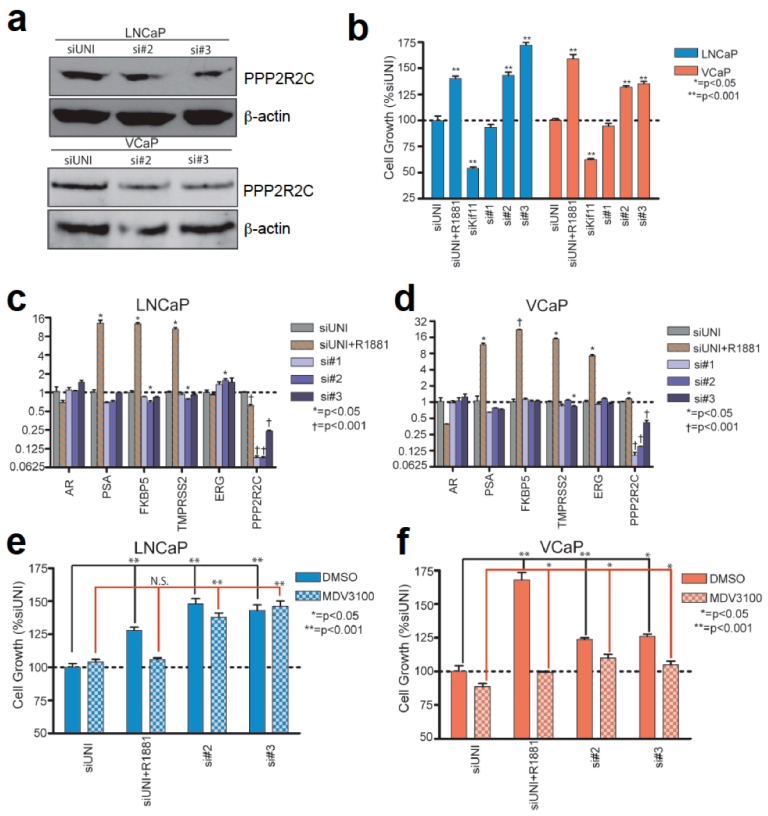

Figure 7. siRNA knockdown of PPP2R2C induces growth in LNCaP and VCaP through pathways independent of the Androgen Receptor. LNCaP and VCaP were transfected with the deconvoluted pool of PPP2R2C siRNAs (si#1-3), in addition to a scrambled control (siUNI) and a positive control for transfection (siKif11). siUNI + 1nM R1881 is a positive control for androgen-induced cell growth and gene expression. (a) Western immunoblot analysis of PPP2R2C protein with siRNAs targeting PPP2R2C. (b) Growth of LNCaP and VCaP cells in androgen-depleted medium is induced by PPP2R2C-targeted siRNA #2 and siRNA #3, but not siRNA #1 (c – d) qRT-PCR analysis of cDNA collected from LNCaP (c) and VCaP (d) demonstrates successful suppression of PPP2R2C expression. PPP2R2C knockdown does not increase the expression of AR or AR-regulated genes (PSA, FKBP5, TMPRSS2, ERG). Co-treatment with non-targeting siRNA + 1nM R1881 induced AR-regulated gene expression. Statistical comparisons of gene expression were performed between siUNI and gene-specific siRNA within each cell line. (e – f) LNCaP (e) and VCaP (f) were transfected with non-targeting siRNA or siRNA targeting PPP2R2C and co-treated with 5μM MDV3100. Raw luminescence values in each cell line were normalized to siUNI. Statistical comparisons between siRNA targeting PPP2R2C and siUNI were performed within each cell line.

3d. Determine the impact on ARIPC growth of inhibition of candidate pathway #2 identified during Tasks 2g and 2h--(Y3Q4) NF

To be completed in Year 3.

3e. Determine the impact on ARIPC growth of inhibition of candidate pathway #3 identified during Tasks 2g and 2h--(Y3Q4)NF

To be completed in Year 3.

Milestone #7: Results demonstrating the impact of inhibition of candidate pathways #1, #2, and #3 on ARIPC xenografts -(Y3Q4)

To be completed in Year 3.

Task 4: Analysis of scientific aim 3: To determine if simultaneously co-targeting the AR pathway and ARIPC survival pathway(s) in AR-sensitive prostate cancers will augment tumor responses and delay/prevent recurrences.

4a.(Revised) Determine the impact of co-targeting AR and the most promising pathways out of SRC, MYC, OxPhos or HDAC in castration sensitive prostate cancers. (Y2Q4)-N

We have completed experiments co-targeting SRC (with Dasatinib) and OxPhos (with DNP) in two CRPC prostate cancer xenografts. Briefly, CB17-SCID mice were castrated at eight weeks of age. After two weeks recovery, these mice were subcutaneously implanted with a LuCaP 35V or LuCaP49 tumor fragments. Mice were measured daily for tumor growth. When a mouse with a tumor achieved a volume of 200 mm^3, it was enrolled into Vehicle, DNP, or Dasatinib treatment groups. Treatments were administered once daily by oral gavage and tumor measurements were conducted every other day. Tumors were harvested from mice when they had a volume greater than 800 mm^3. Paired t-test analysis indicates that there is a significant difference between Vehicle and Dasatinib treatment duration ($P = 0.0457$) with a 95% confidence interval. There does not appear to be a significant difference between Vehicle or Dasatinib treatments compared to DNP treated tumors. This result indicates that although treatments do not eliminate or shrink LuCaP 35V tumors, it takes longer for Dasatinib treated tumors to grow, thus inhibiting castrate resistant tumor cell growth. However, the overall suppression of CRPC tumor growth is quite modest and unlikely to represent a clinically-meaningful response in these two tumor types (LuCaP35V and LuCaP49).

The planned *Task is complete*, however, molecular analyses of the resistant/progressing tumors are underway to assess how effectively Dasatinib inhibiting Src pathway activity.

4b. (Revised) Determine the impact of co-targeting the second most promising pathways out of SRC, MYC, OxPhos or HDAC in castration sensitive prostate cancers.- (Y2Q4)N

See above for results with DNP. *Task complete*.

Milestone #8:(Revised) Results demonstrating the impact of cotargeting AR and either MYC or HDAC inhibition on prostate cancer xenografts. (Y2Q4).

The studies co-targeting MYC and AR are partially complete (see Figure 6 above). These studies have been expanded to include additional CRPC and ARIPC cell lines to determine if there is a consistent effect involving the suppression of Myc transcript/protein or the pharmacological inhibition of Myc and the development or progression of ARIPC. *Task ongoing*.

4c. Determine the impact on hormone naïve prostate cancer xenograft growth of co-targeting candidate pathway #1 and AR (Y3Q3) NF

Studies to be completed in Y3Q3.

4d. Determine the impact on hormone naïve prostate cancer xenograft growth of co-targeting candidate pathway #2 and AR (Y3Q4)NF

Studies to be completed in Y3Q4.

4e. Determine the impact on hormone naïve prostate cancer xenograft growth of co-targeting candidate pathway #3 and AR (Y3Q4) NF

Studies to be completed in Y3Q4.

Milestone #9: Results demonstrating the impact of co-targeting AR and candidate pathway #1, #2, or #3 on prostate cancer Xenografts

Studies to be completed in Y3Q4.

Task 5: Reporting of protocol processes (To begin year 2).

5a. Review and summarize pathways specifically associated with ARIPC (Y2Q2)

Studies to be completed in Y2Q2.

Milestone #10: Abstract submission to AARC annual meeting reporting pathways associated with ARIPC (Y2Q2)

The following abstracts were submitted and presented:

1. Bluemn EG, Annis J, Grandori C, Nelson PS. Exploiting the Genomic Programs Underlying Androgen-Receptor-Null Prostate Cancer. Department of Defense IMPaCT conference. Orlando, FL, March 2011. Poster P6-44.

2. Qu X, Davison J, Bluemn EG, Nelson P, Vessella R, Fang M. Genome-wide methylation analysis in advanced-stage prostate cancer models. AACR-Advances in Prostate Cancer Research Conference, 2012. Orlando, FL, February 2012.

3. Spencer ES, Bluemn EG, Gordon, R, Zhang, X, Johnston, RB, Lucas, J, Nelson, P, Porter, C. Association of decreased expression of protein phosphatase 2A subunit PR55γ (PPP2R2C) with an increased risk of metastases and prostate cancer-specific mortality. ASCO conference. June, 2012.

5b. Prepare manuscript reporting identification and validation of pathways specifically associated with ARIPC (Y2Q3)

The following manuscript was submitted for publication in October 2012

Bluemn EG, Spencer, ES, Mecham, B, Gordon, R, Coleman, I, Lewinshtein, D, Annis, J, Grandori, C, Porter, C, Nelson, PS. PPP2R2C loss promotes castration-resistant prostate cancer growth and is associated with increased prostate cancer-specific mortality. Oncogene. In submission.

Milestone #11: Submit abstract/manuscript reporting identification and validation of pathways associated with ARIPC (Y2Q3)

Task Complete and Milestones Achieved (see 5a and 5b).

5c.Prepare manuscript reporting the impact of inhibiting SRC in castration sensitive and ARIPC prostate cancer xenografts (Y3Q2)

Studies to be completed in Y3Q2.

Milestone #12: Submit abstract/manuscript reporting impact of SRC inhibition in castration sensitive and ARIPC xenografts (Y3Q2)

Studies to be completed in Y3Q2.

5d.Prepare manuscript reporting the impact of inhibiting oxidative phosphorylation in hormone naïve and ARIPC xenografts (Y3Q2)

Studies to be completed in Y3Q2.

Milestone #13: Submit manuscript reporting impact of oxidative phosphorylation inhibition in castration sensitive and ARIPC xenografts (Y3Q2)

Studies to be completed in Y3Q2.

5e. Prepare manuscript reporting on the impact of inhibiting candidate pathways #1, #2, and/or #3 in hormone sensitive and ARIPC xenografts (Y3Q4)

Studies to be completed in Y3Q4.

Milestone #14: Submit abstract/manuscript reporting on the impact of inhibiting candidate pathways #1, #2, and/or #3 in hormone sensitive and ARIPC xenografts (Y3Q4)

Studies to be completed in Y3Q4.

Key Research Accomplishments

- We have completed the laser capture microdissection and q/c of attendant RNA/DNA from 196 castration resistant prostate cancers and matched controls.
- We have completed transcript profiling (including AR expression) from 196 prostate cancer metastasis (those with suitable quality RNA) and matched controls.
- We have completed genome analysis (copy number and sequence analysis) for 196 prostate cancer metastasis (those with suitable quality DNA) and matched controls.
- We have completed a high-throughput screen to identify genes and networks involved in maintaining CRPC growth and potentially bypassing AR signaling for facilitating tumor progression. This screen identified components of the PP2A signaling complex as key factors.
- We have completed preclinical trials of 2 key nodes hypothesized to regulate CRPC growth: OxPhos and Src. The Src inhibitor Dasatinib delayed CRPC growth whereas the OxPhos inhibitor DNP did not inhibit growth.
- We have presented results of these studies at three National meetings of prostate cancer research and submitted a manuscript detailing the PP2A studies for publication.

Reportable Outcomes

1. Bluemn EG, Spencer, ES, Mecham, B, Gordon, R, Coleman, I, Lewinshtein, D, Annis, J, Grandori, C, Porter, C, Nelson, PS. PPP2R2C loss promotes castration-resistant prostate cancer growth and is associated with increased prostate cancer-specific mortality. Oncogene. In submission.
2. Bluemn, EG. Bypassing androgen pathway dependence in advanced prostate cancer. [PhD dissertation]. Seattle, WA: University of Washington; 2012.

3. Spencer ES, Bluemn EG, Gordon, R, Zhang, X, Johnston, RB, Lucas, J, Nelson, P, Porter, C. Association of decreased expression of protein phosphatase 2A subunit PR55γ (PPP2R2C) with an increased risk of metastases and prostate cancer-specific mortality. ASCO conference. June, 2012.
4. Hu R, Lu C, Mostaghel EA, Yegnasubramanian S, Gurel M, Tannahill C, Edwards J, Isaacs W, Nelson PS, Bluemn EG, Plymate SR, Luo J. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. Cancer Res. In press.
5. Qu X, Davison J, Bluemn EG, Nelson P, Vessella R, Fang M. Genome-wide methylation analysis in advanced-stage prostate cancer models. AACR-Advances in Prostate Cancer Research Conference, 2012. Orlando, FL, February 2012.
6. Bluemn EG, Nelson PS. The androgen/androgen receptor axis in prostate cancer. Curr Opin Oncol. 2012 May;24(3):251-7.
7. Bluemn EG, Annis J, Grandori C, Nelson PS. Exploiting the Genomic Programs Underlying Androgen-Receptor-Null Prostate Cancer. Department of Defense IMPaCT conference. Orlando, FL, March 2011. Poster P6-44.

Conclusion

We have completed the proposed year 2 tasks that comprise the specific aims of our proposal. We are on-track to complete year 3 tasks and milestones. The data generated from profiling human CRPC metastasis and shRNA screening studies will now be used to identify additional AR-bypass pathways that plausibly contribute to CPRC growth, and specifically, ARIPC progression.

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